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ORIGINAL RESEARCH

Hylocereus polyrhizus effect on catalase and malondialdehyde in rats with oxidative stress

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Abstract. The excessive production of free radicals causes oxidative damage and degenerative diseases. Whereas, catalase is a macromolecule with a specific activity, but Malondialdehyde (MDA) is a small molecule with no activity. The peels of red dragon fruit (*H. polyrhizus*) are potential to be natural exogenous antioxidants. This research aimed to determine the effects of methanol fraction from *H. polyrhizus* peels on catalase activity and MDA level. The peel was macerated using chloroform and fractionated using methanol. The stress condition was done through five-day fasting and ten-minute swimming per day. The activity of catalase and MDA was measured using UV-Vis spectrophotometer. Thirty five rats were divided into 7 groups: normal, negative, dose I (5 mg/200gBW), dose II (10 mg/200gBW), dose III (20 mg/200gBW), positive control (quercetin) (4 mg/200gBW) and vitamin E (17.64 mg/kg). The activity of catalase in the normal, negative, vitamin E, and quercetin, dose I, II, and II respectively were: 28.37; 7.8; 24.85; 34.33; 59.92; 28.18; and 70.85 Unit/mL. The average MDA level in the groups respectively were: 0.042; 0.051; 0.034; 0.042; 0.037; 0.033; 0.030 µg/mL. The result of One Way ANOVA test showed that the catalase activity in dose III was significantly different than normal group; the MDA levels in all groups were significantly different than normal group. This research revealed that methanol fraction from *H. polyrhizus* peels is a potent antioxidant for its capacity to increase the activity of catalase and reduce the MDA level in rats with oxidative stress.

Keywords: Catalase, *H. polyrhizus*, MDA, Oxidative stress

INTRODUCTION

The demand for fulfilling the life needs was inevitable so that people had to work hard, and it often led to irregular meal and rest time. The hard work without rest would finally make body organs bear burdens and trigger free radicals. Free radicals could be produced from endogenous process as a normal response to either intracel or extracel biochemical process and extracel and exogenous process as a result

of pollution, food and skin injection and absorption. The production of free radicals in body was able to increase oxidative stress condition. In a normal condition, the produced free radicals would be neutralized by antioxidants in the body. The high concentration of free radicals made endogenous antioxidants unable to neutralize free radicals, and this resulted in oxidative stress, an imbalance between free radicals and antioxidants [1]. The indicators used to determine oxidative stress in human body were catalase and malondialdehyde (MDA). Catalase was an enzyme containing heme that catalyzes hydrogen peroxide dismutation to water and oxygen; MDA was lipid peroxidation resulted from free radicals. The oxidative stress induced with swimming and fasting could reduce the activity of catalase in rats' liver, muscle, heart and brain and increase the MDA concentration in rats' liver [2,3], thus antioxidants from outside the body was needed.

The peels of red dragon fruit (H. polyrhizus) contained beta carotene and betacyanin which have antioxidant activity [4,5]. Based on antioxidant activity test using DPPH radical scavenging activity method, methanol extracts from H. polyrhizus peels had higher antioxidant activity than the extracts from white dragon fruit (H. Undatus) peels and from H. polyrhizus flesh [6,7]. Methanol extracts, ethyl acetate soluble fractions, and ethyl acetate insoluble fractions from red dragon fruit peels had antioxidant activity with IC50 241.19 µg/mL; 8.34 µg/mL and 46.84 µg/mL respectively [8]. The antioxidant activity of the fruit peel isolates was 2.952,14 µg/mL [9]. Those researches revealed that the antioxidant activity was tested

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through in vitro method. Therefore, a research to investigate the increase on catalase concentration and measure MDA concentration in male Wistar rats with oxidative stress after injection of methanol fraction from H. polyhizus peels is required.

METHODOLOGY

The tools used in this research were analytical balance (Precisa XB 4200 C®, Precisa XT 220 A®), a set of glass instrument, oven (memmert®), water-bath, desiccator, evaporator (Heidolph®), sonde (Spuit Syringe p.o), minor set, micropipette, microtube, blue tip, microcentrifuge, and UV-Vis spectrophotometer (Shimadzu®).

The materials were *H. polyrhizus* peels, quercetin, vitamin E, chloroform p.a., methanol p.a., phosphate buffered saline (PBS), potassium chloride (KCL), potassium phosphate buffer, and hydrogen peroxide (H2O2), Thiobarbituric acid (TBA), tetramethoxypropane (TMP), HCL, butylated hydroxytoluene (BHT), and Trichloroacetic (TCA).

Collection and Process of Fruit Peels

The fruit was obtained from Petani Mekar Sari Plantation, Segedong Sub-district, Pontianak District, West Kalimantan Province. The fruit peels were washed with flowing water, chopped, and wet sorted. Furthermore, they were dried and put in a blender to generate simplicia passing 40 mesh strainer.

Extraction and Fractionation

The extraction method applied in this research was maceration. The simplicia of *H. polyrhizus* peels was put into a maceration vessel and added with chloroform until all samples got submerged in the solvent; the tube was then closed. The maceration took 7 days. The maceration results were filtered to separate their extract and residue. The generated extract was collected and concentrated with evaporator at 40°C to evaporate the solvent and obtain thick chloroform extract.

It was fractionated in liquid form with methanol. This process resulted in two phases of separation based on the polarity index of methanol fraction and residue fraction. Methanol fraction found on the upper layer was collected and concentrated with waterbath at the temperature to obtain thick methanol fraction from the peels.

Test of Catalase Activity

Preparation of Experimental Animals

Male Wistar rats were used in this research. They were at the age of 2-3 months with average weight of 100-200g. The animals were adapted to the experimental cage for 1 week and classified into 7 experimental groups. Each group consist of 3 rats. Group 1 was normal rats without treatment; group 2 was positive oxidative stress; group 3 was stress oxidative treatment with methanol fraction 5mg/200gBW; group 4 was stress oxidative treatment with methanol fraction 10mg/200gBW; group 5 was stress oxidative treatment with methanol fraction 20mg/200gBW; group 6 was stress oxidative treatment with quercetin 4mg/200grBW; group 7 was stress oxidative treatment with Vitamin E 17.64 mg/kgBW.

The oxidative stress treatment was performed through fasting, without food but with ad libitum water. In addition, the rats were made to swim for 10 minutes per day for 5 days. Methanol fraction, quercetin, and Vitamin E were given orally using sonde. After 5 days of treatment, the rats were terminated by cervical dislocation, after anesthetized with chloroform. The rats were dissected for their livers. The organs were washed with phosphate buffered saline (PBS).

Preparation of Rat Liver Homogenate

Rat livers (1.25g), in 5mL PBS containing 11.5g/L KCL, were chopped and centrifuged with microcentrifuge at 4000 rpm for 10 minutes at 4°C to obtain clear supernatants (homogenate). These were used to analyze the activity of antioxidant enzymes including catalase [10].

Measurement of Catalase Activity

The clear supernatants of rat livers (0.5 mL) were added with 2.0 mL potassium phosphate buffer (50 mM (pH 7.0)) containing 10 mM H2O2. The changing absorptions were measured with UV-Vis spectrophotometer at λ 240 nm, recorded every 15 seconds for 1 minute.

The catalase activity was measured bassed on the hydrogen peroxide reduction; it was calculated using absorption slope curve of the sample solvent and blank solvent with the formula [11].

Catalase Activity (U/mL) = $\frac{(SL-SLb)}{0.0436} \times \frac{2.5}{0.5}$ (1)

Analysis of MDA Concentration

Preparation of Standard Curve

The reagent solvent stock, 1,1,3,3-tetramethoxypropane (TMP) at 6M concentration was diluted to be 0.05; 0.06; 0.07; 0.0; 0.09; 0.10; 0.11; 0.12; and 0.13 ppm. Furthermore, it was added with 2.0 ml HCL (0.25 N) containing 15% TCA, 0.38% TBA and 0.5% BHT. The mixture was heated at 80°C for 1 hour, centrifuged at 3500 rpm for 10 minutes. Its supernatants were collected and measured for their absorbance with spectrophotometer at λ 532 nm [10].

Measurement of Liver MDA

The clear supernatants of rat livers (0.5 mL) were added with 2.0 mL HCL (0.25 N) containing 15% TCA, 0.38% TBA and 0.5% BHT. The mixture was heated at 80°C for 1 hour. Its supernatants were collected and measured for their absorbance with spectrophotometer at λ 532 nm. Tetramethoxypropane (TMP) was used as the standard solvent [11].

Statistical Analysis

One-way ANOVA and Least Significant Difference (LSD) test were applied to analyze the data.

RESULTS AND DISCUSSION

The reduced activity of catalase that occurred in normal group was significantly different form that in negative control ($p<0.05$). This indicated that swimming treatment for 10 minutes and fasting could cause oxidative stress. This was in accordance with the research by Misra, et.al stating that swimming treatment for rats resulted in oxidative stress, and it could significantly reduce the activity of catalase [2]. Oxidative stress was a serious imbalanced between the production of reactive oxygen species (ROS) and the defence of antioxidant. It was able to cause tissue injuries, damage of all molecule targets, DNA, protein, and lipid (lipid peroxidation) [12]. **Figure 1** shows the catalase activity of rats with some treatments.

The principle of MDA measurement was the reaction of one MDA molecule with two Thiobarbituric acid (TBA) molecules which generated pink color measured using spectrophotometer at 532 nm of wavelength.

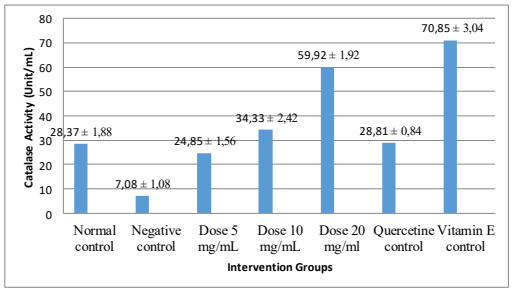

Figure 1. Histogram of Catalase Activities Average of Rat in Each Group. Remark: *= significantly different from normal group, result of One-Way ANOVA $p<0.05$)

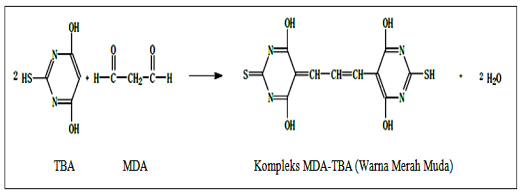

Figure 2. Reaction of Complex TBA-MDA Formation.

The standard used to measure MDA concentrations was tetramethoxypropane (TMP). In acid condition, TMP could be hydrolyzed resulting in hemiacetal and methanol. The produced hemiacetal was then decomposed into methanol and aldehyde which were able to react with TBA [13]. The linear regression equation generated from the standard curve measurement was $y = 8.17993x - 0.09969$ with $r = 0.99268$. **Figure 3** shows the graph of relation between absorbance and standard curve of TMP.

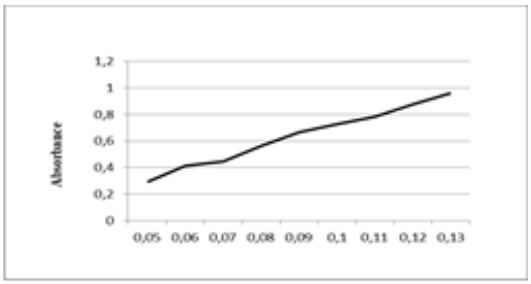

Figure 3. Graph of TMP Standard Curve at λ 525 nm

The result of ANOVA analysis showed that the MDA concentration of negative control was significantly different from those of the other groups ($p<0.05$). This explained that 10-minute swimming and fasting implemented to Wistar rats resulted in oxidative stress due to the higher increased MDA concentration than that in normal condition. The rats induced with swimming and 5-day fasting in negative control group had higher MDA concentration than the normal group did [3,13]. In accordance, this research showed that the negative control group had higher MDA concentration than the normal

group did. **Figure 4** shows the graph of average concentration for each group.

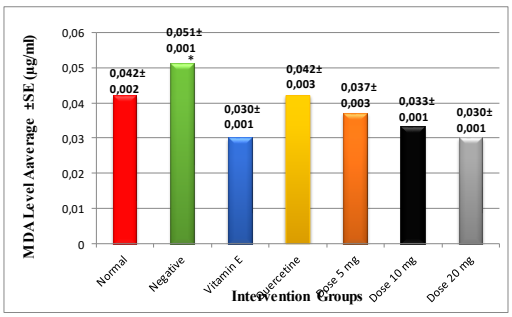

Figure 4. Histogram of MDA Concentration Average of Each Group. Remark: *= significantly different from negative control group, result of One-Way ANOVA $p<0.05$)

The extraction method applied was maceration. The method was selected because it was simple, did not require complex tools, and was able to avoid component damage of compounds which could not bear heat as the method was without heating process. The chloroform extracts from *H. polyrhizus* peels were fractionated with methanol. Accordingly based on preliminary study the chloroform extract generate strong antioxidant activity. Extraction in liquid form was an extraction method based on solubility of the component target and in two separated solvent materials [14]. Enzymatic antioxidants found in body including catalase had an important role to protect body against reactive oxygen species (ROS). It was a term of oxidant groups involving free radicals and molecules able to produce free radicals. ROS included superoxide anion (O2), hydrogen peroxide (H2O2), peroxyl radical (ROO), and hydroxyl radical (OH) [15].

Catalase would catalyze the decomposition of hydrogen peroxide (H2O2) into water and oxygen to protect cells from oxidative damage [2]. The first reaction explained that one molecule of hydrogen peroxide (H2O2) oxidized heme found in catalase in resting-state form (Enz (Por-FeIII)) into oxyferryl. One oxidation was equal to the reduction of iron (Fe) and one of porphyrin (Por) ring to produce compound I (Cpd I) that was porphyrin cation radical (Por+-FeIV=O). Moreover, one molecule of hydrogen peroxide in the second reaction was used as reductor of compound I to regenerate resting-state enzyme (Enz (Por-FeIII)), water (H2O), and oxygen (O2) [12]. The reactions were:

Enz (Por-FeIII) +H2O2 → Cpd I (Por+-FeIV=O)+H2O (2)

Cpd I(Por+-FeIV=O)+H202 → Enz (Por-FeIII)+H2O+O2 (3)

During the physical activities such as swimming, the oxygen consumption of body increased. Rats treated with physical activity in the form of swimming, will be easily suffer from oxidative stress. Under physiologic condition, free radicals, as the result of metabolism, can be overcome by endogenous antioxidant. Increase activity or oxidant will lead to higher need of the antioxidant. Under streesfull condition, catalase will be lessersince this enzyme component as a complex protein has been damaged by free radicals. The excessive radicals would result in oxidative stress condition since endogenous antioxidants were unable to absorb the free radicals [16].

In addition to swimming, rats were fasting but with ad libitum water. Fasting was a condition that could cause oxidative stress. The neutral fats were catabolized into fatty acid and glycerol; fatty acid was the main source of energy production. In a normal condition, the catabolism of fatty acid occurred in the mitochondria through ß-oxidation process. Whereas, in a hunger condition, ß-oxidation process increased in peroxisome that was normally a minor line of the process [17]. The increased activity of ß-oxidation increased number of free radicals, additional result of metabolism process. The addition of methanol extracts (5mg/mL, 10mg/mL and 20mg/mL) from the peels of red dragon fruits could increase the activity of catalase more significantly than that of the negative control group ($p<0.05$). The rats given dose of 5mg/mL and10mg/mL had catalase activity which was not significantly different from that of the normal rats ($p>0.05$). It was better than that of the normal group after the addition of 20mg/mL methanol fractions from the fruit peels.

This was predicted as the effect of secondary metabolites in the peels of red dragon fruit. Kim stated that the peels of red dragon fruit contained polyphenols and flavonoids [6]. The

fruit peels contained beta carotenes and betalains [4]. Polyphenols in the peels were considered to increase the activity of catalase since the compounds could be antioxidant. The addition of polyphenolic compounds from *Halimeda opuntia* (Linnaeus) Lamouroux could increase the activity of catalase in rat liver induced with carbon tetrachloride [18]. It was considered as the effect of polyphenols that could influence the transcription reaction in expression process of catalase gene [18]. Moreover, another antioxidant compound that could increase the catalase activity was flavonoid. The increase of catalase activity in hepatic cells of human after the addition of flavonoids was daidzein [18]. The compunds were considered able to induce the transcription of catalase gene through interaction with the part of the gene promotor [19].

Another compound functioning as antioxidant in methanol fractions from the red dragon peels was betalain included in secondary metabolite of phenolics. The extracts of *Cyclea peltata* containing alkaloid tetrandrine (Tet), fangchinoline, and coclaurine had capability to increase the activity of antioxidant enzymes such as catalase, superoxide dismutase (SOD), and glutathione (GSH) in rats induced with carbon tetrachloride (CC14) [20]. Metals such as copper and iron found in biological system would interact with H2O2 through enton reaction to produce hydroxyl radical (OH-). It was able to induce the damaged DNA chain and cells. The alkaloids found in methanol fraction from the peels of red dragon fruit functioned as antioxidant; they played a role as scavangers of hydroxyl radical (OH-) and ion chelators [21,22].

Beta carotene found in methanol fractions from the red dragon peels also functioned as antioxidant. The addition of beta caroteneswas able to increase the activity of catalase in rats induced with acetaminophen [23]. The compound was considered as antioxidant since it could increase gene expression of antioxidant enzymes which were catalase and superoxide dismutase (SOD) by enhancing the number of mRNA [24].

The positive control used was quercetin. It was considered able to detoxify H2O2 and inhibit the ROS production through fenton reaction to generate hydroxyl radical with ion chelators of transition metals such as Cu2+ [25]. Antioxidant activity of quercetin showed that the compound was able to inhibit DNA damage and alkaline oxidation of pyrimidine in cells induced with hydrogen peroxide, free radical [26].

Furthermore, Vitamin E was also used as the positive control. The catalase activity in Vitamin E group was significantly different from those of the other groups ($p<0.05$). Vitamin E or alpha-tocopherol was used to inhibit oxidative damage by stopping the propagation process of oxidation of double-bond unsaturated fatty acid [27]. Vitamin E was considered able to control hydrogen peroxide production directly in mitochondria, and the excessive production of ROS in mitochondria was the main cause of the injured tissues in rats deficient in Vitamin E [28].

The ability of methanol fractions from the peels of red dragon fruit to increase the activity of catalase in rats with oxidative stress was not as good as that of positive control, Vitamin E. this was due to the unidentified exact number of secondary metabolite found in methanol fraction functioning to increase the catalase activity. Nevertheless, the fraction remained potential to be antioxidant, indicated by the increased catalase activity in rats given methanol fraction compared with the goups of negative control, normal control, and positive control (quercetin).

Moreover, the data analysis of positive control group (Vitamin E) showed that the MDA concentration in this group was significantly different form that of the negative control ($p<0.05$). The average of MDA concentrations was 0.034 µg/ml. Vitamin E functioned as chain breaking antioxidants inhibiting the propagation stage by one hydrogen ion donor from 6- hydroxyl group of chroman ring able to change peroxyl radicals, result of oxidized lipids, into less reactive tocopherol radicals which was unable to break the fatty acid chain [29]. Tocopherol scavenged peroxyl radicals faster than peroxyl radicals to react with lipid substrate did. This compound would be stable free radicals (tocopheroxyl radical). The reaction of tocopheroxyl radicals with peroxyl radicals would generate two stable products

which were α–tocopherylquinone and epoxyquinone.

The reduced MDA concentration in quercetin group was significantly different from that of the negative control group ($p<0.05$). The MDA concentration of the first group was 0.042 µg/ml. Quercetin had antioxidant effects so that it could reduce the MDA concentration in rats with oxidative stress. In adiition, it was able to reduce the MDA concentration in Wistar rat gastric induced with ethanol.

The Least Significant Difference (LSD) test, dose I, II, and III groups had reduced MDA concentration significantly different from that of negative control ($p<0.05$). The reduced MDA concentration, however, was not significantly different from those of normal control, positive control Vitamin E, and quarcetin. This indicated that the three doses of methanol fractions from the furit peels had antioxidant effects. *H. polyrhizus* contained secondary metabolites which were beta carotenes and betacyanins. Kim showed that methanol extracts from the peels of *H. polyrhizus* contained secondary metabolites which were polyphenols and flavonoids [6]. The metabolites were considered responsible for the antioxidant activities. The peels of *H. polyrhizus* contained beta carotenes, terpenoids types of carotenoids which had antioxidant activities [4]. Carotenoids were antioxidant with a role as free radical breaker. It reacted with peroxyl radicals, result of oxidized lipids, and resulted in less reactive antioxidant radicals to restart the propagation process of free radicals. The formation of free radicals could be terminated when it rected with other radicals by forming stable products to break the free radical chains.

CONCLUSION

The methanol fractions from the peels of *H. polyrhizus* with doses of 5mg/200gBW, 10mg/200gBW and 20mg/200gBW were able to increase the activities of catalase in rats with oxidative stress and reduce the MDA concentrations.

REFERENCE

[1] Winarsih H. 2007. *Natural antioxidants and free radicals.* (Yogyakarta: Penerbit Kanisius)

[2] Barcelos R. P.; Royes L. F. F; Gonzalez-Gallego J.; Bresciani G. 2017. Oxidative stress and inflammation: liver responses and adaptations to acute and regular exercise. *Free Radic. Res.* **51** (2) : 222 – 236.

[3] In S.; T. W.; A. S. 2013. Response of oxidative stress and isoflavone treatment on superoxide dismutase enzyme activities and lipid peroxidation in rat's liver. *Jurnal Ilmu Ternak dan Veteriner*. **18** (2).

[4] Wiset L.; N. Poomsa-ad; V. Srilaong. 2012. Comparisons of antioxidant activity and bioactive compounds of dragon fruit peel from various drying methods. *World Acad Sci Eng Technol.* **70** 995 – 998.

[5] Jamilah B.; Shu C. E.; Kharidah M.; Dzulkifly M. A.; Noranizan A. 2011. Physico-chemical characteristics of red pitaya (*Hylocereus polyrhizus*) peel. *Int. Food Res. J.* **18** 279 – 286.

[6] Kim H.; Choi H. K.; Moon J. Y.; Kim Y. S.; Mosaddik A.; Cho S. K. 2010. Comparative antioxidant and antiproliferative activities of red and white pitayas and their correlation with flavonoid and polyphenol content. *J. Food Sci*. **76** (1) C38-C45.

[7] Nurliyana R.; Syed Z. I.; Mustapha S. K.; Aisyah M. R.; Kamarul R. K. 2010. Antioxidant study of pulp and peel dragon fruits: a comparative study. *Int. Food. Res. J.* **17** 365 – 375.

[8] Wahdaningsih S.; Wahyuono S.; Riyanto S.; Murwanti R. 2017. The radical scavenging activity of 2-2' diphenyl -1-picrylhydrazil (DPPH) on the methanol extracts and Ethyl acetate fractions of red dragon fruit peel (*Hylocereus polyrhizus* (F.A.C.Weber) Britton dan Rose). *nt. J. Phytomedicine* **9**(1) 79.

[9] Wahdaningsih S,; Wahyuono S.; Riyanto S.; Murwanti R. 2018. Antioxidant activity of red dragon fruit peel (*Hylocereus polyrhizus* (f.a.c. weber) britton and rose) isolates using 2,2-diphenyl-1-picrylhydrazyl method. *Asian J. Pharm. Clin. Res.***11**(1) 124.

[10] Moelyono F. S.; Pangkahila W. 2018. Administration of deep sea shark liver oil reduce malondialdehid (mda) levels on male wistar rats exposed to cigarette smoke. *IJAAM* **2** (2) : 28 – 31.

[11] Samarghandian S.; Asadi-Samani M.; Farkhondeh T.; Bahmani M. 2016. Assessment the effect of saffron ethanolic extract (*Crocus sativus* L.) on oxidative damages in aged male rat liver. *Der Pharmacia Lett.* **8** (3) 283 – 290.

[12] Ighodaro O.; Akinloye O. 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria J. Med.*. **54** (4) 87–93.

[13] Mahdi C.; Aulaniam A. 2011. Yogurt Supplementation In Rats (Rattus Norvegicus) Foods Affected by Formaldehyde Exposure to Enzymatic Antioxidant Activity at Hepar tissue. *Natural-B* **1** (2) : 182 – 187

[14] Ministry of health of the Republic Indonesian. 1986. *Galenic preparations*. (Jakarta : Ministry of health of the Republic Indonesian)

[15] Kunwar A.; K. I. Priyadarsini. 2011. Free radicals, oxidative stress and importance of antioxidants in human health. *J. Med. Allied Sci.* **1** (2) : 53 – 60.

[16] Supriyatin.; Rahayu S.; Apriana R. 2017. The effect of maximum physical activity and manggong bamboo (*Gigantochloa manggong*) leaf extract on catalase activity in liver organ of rats (*Rattus norvegicus*). *Metamorfosa: J. Biol Sci*. **4** (1) 8 – 12.

[17] Guyton C. A. 1992. *Buku Teks Fisiologi Kedokteran*. (Jakarta : Penerbit Buku Kedokteran EGC)

[18] Ana Mara De Oliveira E. Silva; Vidal-Novoa A.; Batista-González A. E.; Pinto J. R.; Mancini D. A. P.; Reina-Urquijo W.; et. al. 2012. In vivo and in vitro antioxidant activity and hepatoprotective properties of polyphenols from Halimeda opuntia (Linnaeus) Lamouroux. *Redox Report.* **17** (2) 47 – 53.

[19] Krych J.; Gebicka L. 2013. Catalase is inhibited by flavonoids. *Int. J. Biol. Macromol.* **58** 148 – 153.

[20] Shine V. J.; Latha P. G.; Suja S. N. R.; Anuja G. I.; Raj G.; Rajasekharan S. N. 2014. Ameliorative effect of alkaloid extract of Cyclea peltata (Poir.) Hook. f. & Thoms. roots (ACP) on APAP/CCl4 induced liver toxicity in Wistar rats and in vitro free radical scavenging property. *Asian Pac. J. Trop. Biomed.* **4** (2) 143 – 151.

[21] Moura DJ.; Richter MF.; Boeira JM.; Henriques JAP.; Saffi J. 2007. Antioxidant properties of -carboline alkaloids are related to their antimutagenic and antigenotoxic activities. *Mutagenesis*. **22** (4) 293 – 302.

[22] Jang MH.; Kim HY.; Kang KS.; Yokozawa T.; Park JH. 2009. Hydroxyl radical scavenging activities of isoquinoline alkaloids isolated from Coptis chinensis. *Arch. Pharm. Res.* **32** (3) 341 – 5.

[23] Morakinyo AO.; Iranloye BO.; Oyelowo OT.; Nnaji J. 2012. Anti-oxidative and hepatoprotective effect of beta-carotene on acetaminophen-induced liver damage in rat. *Biol. Med.* **4** (3) 134.

[24] Júnior JVR.; Araújo GR.; Pádua BDC.; Magalhães CLDB.; Chaves MM.; Pedrosa ML, et al. 2012. Annatto extract and β-carotene enhances antioxidant status and regulate gene expression in neutrophils of diabetic rats. *Free Radic. Res*. **46** (3) 329 – 38.

[25] Kim GN.; Jang HD. 2009. Protective mechanism of quarcetin and rutin using glutathione metabolism on HO-induced oxidative stress in hep G2 cells. *Ann N Y Acd Sci.* **1171** - 530.

[26] Zheng Y-Z.; Deng G.; Liang Q.; Chen D-F.; Guo R.; Lai R-C. 2017. Antioxidant activity of quercetin and its glucosides from propolis: a theoretical study. *Sci. Rep* **7** (1) 1 – 11.

[27] Bharrhan S.; Chopra K.; Rishi P. 2010. Vitamin E Supplementation Modulates Endotoxin-induced Liver Damage in a Rat Model. *Am. J. Biomed. Sci.* **51** – 62.

[28] Napolitano G.; Fasciolo G.; Meo SD.; Venditti P. 2019. Vitamin E supplementation and mitochondria in experimental and functional hyperthyroidism: a mini-review. *Nutrients* **11** (12) 2900.

[29] Pokorni J.; Yanishlieva N and Bordon M. 2001. *Antioxidant in food,* Pratical Apllication. (New York:C&C Press).